UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF OMEGA-3 POLYUNSATURATED FATTY ACID ENRICHED CHEVON USING TREATED OIL PALM (ELAEIS GUINEENSIS JACQ.) FROND SILAGE

MAHDI EBRAHIMI

FPV 2012 17

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By

MAHDI EBRAHIMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2012

DEDICATION

MY FATHER AND MOTHER,
I Love You Forever

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: **Professor Mohamed Ali Rajion, PhD**

Faculty: **Veterinary Medicine**

There is a need to treat oil palm fronds (OPF) to improve its nutritive value and digestibility to be used as small ruminant feed. A study was carried out in which fresh OPF have been ensiled with lactic acid bacteria (LAB), cellulase enzyme and a combination of LAB and cellulase enzyme to improve the fermentation quality and nutrient values of the OPF. The dietary treatment methods comprised non-ensiled fresh OPF (F), ensiled OPF with no additives (C, control), ensiled OPF with a commercial lactic acid bacteria (LAB) inoculant (I), ensiled OPF with an exogenous fibrolytic, cellulase enzyme (E) and ensiled OPF with a LAB inoculant-cellulase combination (I+E). Ensiling was carried out with about 2 Kg samples stored in air-tight glass jars at 25-30 ^{o}C for 12 wk after which the silage samples were subjected to standard proximate analyses. The ensiling decreased significantly ($P < 0.05$) the DM concentration of ensiled OPF with additives compared to the non-ensiled fresh OPF and control silage. The LAB inoculant-cellulase combination reduced significantly ($P < 0.01$) the NDF and ADF content compared to the control silage. Crude fat also decreased significantly ($P < 0.05$) after ensiling compared to the non-ensiled fresh OPF. All the treated silages had a lower pH (4.09-4.28) than the untreated silage

(4.88). Acetic acid and propionic acid in the ensiled OPF were significantly ($P < 0.01$) higher than the non-ensiled fresh OPF. The NH_3-N concentration in the silage with additives significantly decreased ($P < 0.01$) compared to the non-ensiled fresh OPF and control silage. The LAB inoculant-cellulase combination increased the water soluble carbohydrate (WSC) and ethanol production significantly ($P < 0.01$) compared to the control silage. The population of LAB increased significantly ($P < 0.05$) in all treatments with additives compared to the non-ensiled fresh OPF and control silage.

The *in vitro* dry matter digestibility, *in sacco* dry matter digestibility, *in vivo* dry matter digestibility, *in vitro* gas production, *in vitro* fermentation kinetics and *in vivo* fermentation kinetics of the silages were determined using rumen fistulated goats. The OPF silage treated with cellulase or the LAB inoculant-cellulase combination showed a significantly ($P < 0.01$) higher *in vitro* gas production and dry matter digestibility (DMD) compared to the non-ensiled fresh and control silage. In fact, the *in vitro* gas production, including maximum gas production and the rate of gas production of the ensiled OPF were all increased by the treatment with either cellulase or the LAB inoculant-cellulase combination.

The OPF silage treated with the LAB inoculant-cellulase combination showed significantly higher ($P < 0.01$) rapidly soluble fractions (18.23%), insoluble but fermentable fractions (43.86%) ($P < 0.05$) and fractional degradation rates (0.018 h^{-1}) ($P < 0.05$) than the other treated silages.

The *in vivo* dry matter digestibility of the OPF silage with the LAB inoculant-cellulase combination was 4.12% higher ($P < 0.01$) than the control silage. Thus, the ensiled OPF treated with the LAB-cellulase combination was selected to be the best treated OPF to be used in the animal feeding trial.

Ruminant products are known to contain high saturated fatty acids (SFA) and low polyunsaturated fatty acids (PUFA), compared to pork, chicken meat or fish. Long chain n-3 PUFA are important in the prevention of modern diseases such as cardiovascular diseases, obesity and cancer. A part of the study attempted to decrease the SFA and increase n-3 PUFA in the chevon using the best treatment of OPF using diets with different n-6: n-3 PUFA ratios. Isonitrogenous and isocaloric experimental diets containing the best treated OPF silage were formulated and either sunflower, palm kernel or linseed oil were used to adjust the dietary n-6: n-3 fatty acid ratios (FAR) to be 2.27:1 (LR), 5.01:1 (MR), and 10.38:1 (HR). Twenty-one five-month old male Boer goats weighing 13.66 ± 1.07 Kg (mean initial body weight ± standard error) were allocated randomly to the three dietary treatment groups and fed for 100 days. The *longissimus dorsi* (LD) muscle, liver, rumen fluid and rumen digesta were sampled at slaughter.

The average daily gain (ADG) for all the experimental groups was similar ($P > 0.05$). The dressing percentage, back fat thickness and rib eye area of the LR group were significantly ($P < 0.05$) higher than the HR group.

The thiobarbituric acid reactive substance (TBARS) values for the LD muscle for all treatment groups after a 6-day post-mortem aging period were significantly ($P < 0.05$) higher than at day 1 and the highest TBARS value belonged to the LR group compared to the HR treatment group after a 6-day post-mortem aging period.

Goats fed the LR diet had a higher ($P < 0.01$) concentration (14.55mM/L) of total VFA in the rumen fluid compared to the HR diet (11.67mM/L).

Increasing the dietary n-6: n-3 FAR lowered ($P < 0.01$) the concentration of C18:0 but linearly increased ($P < 0.01$) C16:0, C18:1 trans-11, C18:2n-6, and cis-9 trans-11 CLA in the liver. The LR diet increased significantly ($P < 0.01$) C18:3n-3, C20:5n-3; C22:5n-3 and C22:6n-3, consequently decreasing the liver n-6: n-3 FAR compared to the HR group. Increasing the dietary n-6: n-3 FAR increased ($P < 0.01$) the C20:4n-6 while the low n-6: n-3 FAR increased ($P < 0.01$) α-linolenic acid (C18:3n-3) concentration in the LD muscle. The C20:5n-3, C22:5n-3 and C22:6n-3 showed positive linear effects ($P < 0.01$) with decreasing the dietary n-6: n-3 FAR.

There was a linearly increased population of cellulolytic bacteria including the *Ruminococcus albus* ($P < 0.05$) and *Ruminococcus flavefaciens* ($P < 0.05$) in the rumen liquid from the LR group compared to the HR group. The population of *Butyrivibrio fibrisolvens* was lower ($P < 0.05$) in the LR group, which had decreased by 31.67%, compared to the HR group.

There was a higher ($P < 0.05$) PPARα and PPARγ gene expression in the LD muscle for the LR group compared with the HR group, indicating that the low dietary n-6: n-3 FAR upregulated the PPARα and PPARγ genes. On the contrary, the stearoyl-CoA desaturase (SCD) gene expression showed a significant ($P < 0.05$) reduction in the LR group compared to the HR group suggesting that the SCD gene was downregulated by the LR dietary treatment.

In conclusion, ensiling the OPF with the LAB inoculant-cellulase combination improved the nutritional value and digestibility of the OPF. Diets formulated incorporating the treated OPF and adjusted to have a low dietary n-6: n-3 FAR (2.27:1) did not adversely affect the growth performance of the Boer goats. In fact the

chevon was enriched with higher proportions of the n-3 PUFA. The increased n-3 fatty acids in the rumen fluid, rumen digesta, liver and LD was suggestive of a reduced biohydrogenation possibly due to changes in the ruminal environment which included a lowered pH, increased VFA concentrations and a drastic decrease in the *Butyrivibrio fibrisolvens* population, the microbe which is actively involved in biohydrogenation in the rumen. The impact of the results derived from this study include the potential reduction in feed costs using the treated OPF as small ruminant feed and the 'healthier' omega-3 enriched chevon would be very appealing to the health-conscious consumer.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN DAGING KAMBING TERPERKAYA ASID LEMAK POLITAKTEPU OMEGA-3 MENGGUNA SILAJ TERPELAKU PELEPAH KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

MAHDI EBRAHIMI

November 2012

Pengerusi : Profesor Mohamed Ali Rajion, PhD

Fakulti : Perubatan Veterinar

Adalah perlu untuk memperlakukan pelepah kelapa sawit (OPF) supaya dapat meningkatkan nilai nutrien dan kebolehcernaannya untuk diguna sebagai makanan ruminant kecil. Kaedah perlakuan ini merangkumi OPF segar bukan ensilaj (F), OPF ensilaj tanpa bahan tambahan (C, kawalan), OPF ensilaj dengan inokulan bakteria asid laktik komersial (LAB), OPF ensilaj dengan enzim fibrolisis eksogenus (selulase) (E) dan OPF ensilaj dengan gabungan inokulan LAB-selulase (I+E). Pengensilajan dilakukan dengan menyimpan lebihkurang 2 Kg sampel dalam balang kaca kedap udara pada 25 - 30°C selama 12 minggu selepas mana sampel silaj ini menjalani analisa proksimat. Pengensilajan mengurangkan secara tererti ($P < 0.05$) kepekatan DM OPF ensilaj dengan bahan tambahan berbanding OPF segar and silaj kawalan. Gabungan inokulan LAB-selulase mengurangkan secara tererti ($P < 0.01$) kandung NDF dan ADF berbanding silaj kawalan. Lemak kasar juga menurun secara tererti ($P < 0.05$) selepas penensilajan berbanding OPF segar. Semua silaj terperlaku menunjukkan pH (4.09-4.28) yang lebih rendah daripada silaj tidak diperlaku (4.88). Asid asetik dan propionik dalam OPF silaj lebih tinggi tererti ($P < 0.01$) daripada OPF

segar. Kepekatan NH_3-N dalam silaj dengan bahan tambahan menurun secara tererti ($P < 0.01$) berbanding OPF segar dan silaj kawalan. Gabungan inokulan LAB-selulase meningkatkan karbohidrat larut air (WSC) dan penghasilan etanol secara tererti ($P < 0.01$) berbanding silaj kawalan. Populasi LAB meningkat secara tererti ($P < 0.05$) dalam semua perlakuan dengan bahan tambahan berbanding OPF segar dan silaj kawalan.

Kebolehcernaan bahan kering *in vitro*, kebolehcernaan bahan kering *in sacco*, kebolehcernaan bahan kering *in vivo,* penghasilan gas *in vitro*, kinetik penapaian *in vitro*, dan kinetik penapaian *in vivo* silaj ditentukan mengguna kambing berfistula rumen. Silaj OPF terperlaku dengan selulase atau gabungan inokulan LAB-selulase menunjukkan penghasilan gas *in vitro* dan kebolehcernaan bahan kering (DMD) lebih tererti ($P < 0.05$) daripada silaj segar dan kawalan. Malah, penghasilan gas *in vitro*, termasuk penghasilan gas maksimum dan kadar penghasilan gas OPF silaj meningkat dengan perlakuan dengan selulase atau gabungan inokulan LAB-selulase.

Silaj OPF terperlaku dengan gabungan inokulan LAB-selulase menunjukkan lebih tinggi tererti pecahan larut pantas (18.23%) ($P < 0.01$) pecahan tak larut tetapi boleh tapai (43.86%) ($P < 0.05$) dan kadar penguraian pecahan (0.018 h^{-1}) ($P < 0.05$) daripada silaj terpelaku lain.

Silaj OPF yang diperlaku dengan gabungan inokulan LAB-selulase menunjukkan penghasilan asid lemak meruap (VFA) keseluruhan lebih tinggi tererti ($P < 0.01$) selepas 3 jam pascapemberian makan berbanding silaj kawalan. Propionat meningkat secara tererti ($P < 0.01$) selepas 3 jam pascapemberian makan dengan silaj berbanding

silaj kawalan. Pengambilan makanan juga meningkat secara tererti ($P < 0.05$) apabila diperlakuan silaj OPF dengan selulase atau gabungan inokulan LAB-selulase berbanding silaj kawalan. Kebolehcernaan bahan kering *in vivo* silaj OPF dengan gabungan inokulan LAB-selulase adalah 4.12% lebih tinggi ($P < 0.01$) daripada silaj kawalan. Justeru, silaj OPF yang diperlaku dengan gabungan LAB-selulase telah dipilih sebagai OPF terperlaku terbaik untuk diguna pada haiwan dalam percubaan pemberian makan haiwan.

Diet isonitrogenus dan isokalori ujikaji mengandungi silaj OPF terperlaku terbaik dirumuskan dan sama ada bunga matahari, isirong sawit atau minyak linseed diguna untuk menyelaraskan nisbah n-6; n-3 asid lemak (FAR) kepada 2.27;1 (LR), 5.01:1 (LR), dan 10.38:1 (HR) dalam diet. Dua puluh satu ekor kambing Boer jantan berumur lima bulan berat badan 13.66 ± 1.07 Kg (min permulaan berat badan ± ralat piawai) diagihkan secara rawak kepada tiga kumpulan perlakuan diet dan diberi makan selama 100 hari. Pada masa penyembelihan karkas dipecahkan kepada dua bahagian (kanan dan kiri). Otot *longissumus dorsi* (LD), hati, bendalir rumen dan digesta rumen disampel. Otot dipek vakum dan disesuaikan selama 1, 3 dan 6 hari dalam pendingin pada 4°C.

Purata kenaikan harian (ADG) untuk semua kumpulan ujikaji adalah serupa ($P > 0.05$). Berat badan haiwan pada penyembelihan dalam kumpulan LR (26.83 Kg) dan MR (26.19 Kg) adalah tinggi sedikit daripada kumpulan haiwan HR (25.68 Kg). Tiada perbezaan tererti ($P > 0.05$) didapati di antara semua kumpulan perlakuan dari segi ciri karkas, sifat dan mutu daging. Bagaimanapun peratus lapah, tebal lemak belakang dan kawasan mata rusuk untuk kumpulan LR adalah lebih tinggi tererti ($P < 0.05$) daripada

kumpulan HR. Parameter mutu daging lain termasuk warna daging, kehilangan memasak, kehilangan drip dan kelembutan tidak berbeza tererti ($P > 0.05$) antarqa semua kumpulan perlakuan.

Nilai bahan reaktif asid tiobarbiturik (TBARS) otot LD bagi semua kumpulan perlakuan selepas 6 hari tempoh penuaan post-mortem adalah lebih tinggi tererti ($P < 0.05$) daripada hari 1, dan nilai TBARS tertinggi berlaku pada kumpulan LR berbanding kumpulan perlakuan HR selepas tempoh 6 hari penuaan post-mortem.

Purata pH bendalir rumen untuk kumpulan LR (6.08) tidak rendah tererti ($P > 0.05$) daripada kumpulan HR (6.39).

Populasi protozoa rumen didominasi oleh Entodinium (95.12 %) dan kurang sedikit oleh spesies Holotrich (4.87 %) yang diberi makan diet LR menunjukkan kepekatan VFA keseluruhan (14.55mM/L) lebih tinggi bererti ($P < 0.01$) dalam bendalir rumen berbanding diet HR (11.67mM/L).

Digesta rumen daripada kambing yang diberi makan diet LR mengandungi perkadaran C18:0 lebih tinggi (7.86 %) berbanding dua lagi kumpulan perlakuan. Pengurangan n-6:n-3 FAR dalam diet menurunkan secara tererti ($P < 0.01$) C18:2n-6 dan cis-9 trans-11 asid linoleik terkonjugat (CLA). Jumlah PUFA n-3 keseluruhan dalam kumpulan LR meningkat secara tererti ($P < 0.01$) berbanding kumpulan HR.

Peningkatan n-6:n-3 FAR dalam diet menyebabkan peningkatan tererti ($P<0.001$) C20:4n-6 sambil n-6:n-3 FAR yang rendah itu meningkatkan ($P < 0.01$) kepekatan α-asid linolenik (C18:3n-3) dalam otot LD kumpulan LR berbanding kumpulan HR.

Akhirnya C20:5n-3, C22:5n-3 dan C22:6n-3 menunjukkan kesan linear positif ($P < 0.01$) dengan penurunan n-6:n-3 FAR.

Penyataan gen PPARα dan PPARγ dalam otot LD dalam kumpulan LR lebih tinggi ($P < 0.05$) berbanding kumpulan HR, menunjukkan n-6:n-3 FAR rendah dalam diet telah menaik regulasi gen PPARα dan PPARγ. Disebaliknya, penyataan gen stearoil-KoA desturase (SCD) menunjukkan penurunan tererti ($P < 0.05$) untuk kumpulan LR berbanding kumpulan HR, menyarankan gen SCD telah diturun regulasi oleh perlakuan diet LR.

Populasi bakteria selulolisis termasuk *Ruminococcus albus* ($P < 0.05$) dan *Ruminococcus flavefaciens* ($P < 0.05$) meningkat secara linear dalam bendalir rumen kumpulan LR berbanding kumpulan HR. Populasi *Butyrivibrio fibrosolvens* adalah lebih rendah ($P < 0.05$) dalam kumpulan LR yang menurum sebanyak 31.67 % berbanding kumpulan HR.

Kesimpulannya, mengensilaj OPF dengan gabungan LAB-selulase meningkatkan nilai nutrien dan kebolehcernaan OPF. Diet yang dirumus dengan memasukkan OPF terperlaku dan tersesuai untuk mengandungi n-6:n-3 FAR (2.27:1) rendah tidak memberi kesan buruk terhadap prestasi pertumbuhan, ciri karkas dan mutu daging kambing Boer sedang membesar. Malah daging kambing menjadi terperkaya dengan perkadaran n-3 PUFA lebih tinggi. Peningkatan asid lemak n-3 dalam bendalir dan digesta rumen, hati dan otot LD menyarankan biopenghidrogenan yang kurang, sebahagiannya disebabkan oleh perubahan dalam persekitaran rumen termasuk pH lebih rendah, peningkatan kepekatan VFA dan penurunan mendadak populasi

Butyrivibrio fibrosolvens yang terlibat dalam biopenghidrogenan dalam rumen. Impak keputusan daripada kajian ini termasuk potensi pengurangan kos makanan menggunakan OPF terlaku sebagai makanan ruminan kecil dan daging kambing terperkaya omega-3 akan menjadi lebih menarik untuk pelanggan yang mementingkan kesihatan.

ACKNOWLEDGEMENTS

First and foremost, my honest appreciation to my supervisory committee, who were involved in my training towards obtaining this degree. I am most grateful to Professor Dr. Mohamed Ali Rajion, the chairman of my supervisory committee for his patience, tireless support, willingness to help, encouragement, kindness and guidance throughout the research and during the preparation of the thesis. I am very much indebted to the members of my supervisory committee namely Associate Professor Dr. Goh Yong Meng, Dr. Awis Qurni Sazili from the Faculty of Agriculture, UPM and Dr. Thomas Schonewille from the Faculty of Veterinary Medicine, Utrecht University, The Netherlands for their encouragement, constructive discussion, excellent advice, comments, and suggestions throughout the project. Special thanks to Professor Dr. Rasedee Abdullah for help with the Bahasa Malaysia abstract translation.

I would like to extend my deepest and sincere appreciation to Universiti Putra Malaysia who supported my candidature and the Ministry of Science and Technology and Ministry of Agriculture and Agro-Based Industry, Malaysia for the E-Science Research Grant which supported the study.

I wish to express my sincere gratitude to the Deans of the Faculty of Veterinary Medicine and Faculty of Agriculture and Directors of the Institute of Bioscience, Institute of Tropical Agriculture, Universiti Putra Malaysia for the use of their facilities and the unlimited assistance from their staff during the course of this study. I would also like to thank staff members of the Veterinary Physiology,

Clinical Pathology and Nutrition Laboratories in particular Mr. Kufli Che Nor, Mrs. Zainab Nasri and Mrs. Rosmawati Hanipah, for their technical assistance. I would like to thank all MARDI staff including Mr. Yunus Ismail and Mr. Fadil Jantan who helped me in the provision of OPF, preparation of silage and pelleting of the feed.

I will always cherish the friendship and help from fellow graduate students Ms Ai Li Ta, and Dr. Suria Kumari.

I would also like to thank all of my past Professors from the University of Tehran in Iran who have encouraged me well and I will be forever thankful. I want to express my huge thanks to my closest friend Dr. Abdoreza Soliemani Farjam who found for me this opportunity to continue my postgraduate education in UPM. I also must thank my closest friends in Kuala Lumpur; Dr. Mohamadreza Sanaii, Dr. Arash Javanmard, Dr. Mohammad Feseleh Jahromi, Dr. Ehsan Oskouian, Dr. Elham Maroufian, Dr Hadi Hajarian, Dr Toktam Hajjr, Parvaneh Mehrbod, Ramin Jorfi, Dr. Hasliza Abu Hassim, Saied Nikbin, Dr. Tang Siew Ching, Su Ting Yong, Hanini Sahari, Dr. Azhary AE Sherydane, Dr. Ainul Yuzairi, Zuriiyaatii Ramly, Aisha Yusuf and back home in Tehran; Seyed Mehdi Khatami, Seyed Javad Mirabdollahi, Bahman Jalali, Seyed Ahmadreza Fakharzadeh, Dr. Hamid Dorodian, Dr. Mostafa Sadeghi and Dr. Reza Taherkhani. I would also like to thank my Heavenly Father (Bahram) and Mother (Sakineh) for providing so many opportunities and support for both my physical and spiritual growth. They have strengthened me during the times when I thought I could go no further. Without them, none of this would have been possible. I also thank all my brothers (Amirhossein and Gholamreza) and sisters (Marzieh, Mahdieh and Hadiseh) for their love, understanding and support.

I certify that a Thesis Examination Committee has met on to conduct the final examination of Mahdi Ebrahimi on his Doctor of Philosophy thesis entitled "Production Of Omega-3 Polyunsaturated Fatty Acid-Enriched Chevon Using Treated Oil Palm Fronds" in accordance with Universities and Unievrsity Colleges Act 1971 and the Constitutions of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy (PhD).

Members of the Examination Committee are as follows:

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Putra Malaysia
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Mohd Jalan, PhD

Agricu
Putra Mal
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Ali Rajion, PhD

Veterinary Medicine
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rskov, PhD

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Exami

SEOW HENG FONG, PHD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Mohamed Ali Rajion, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Goh Yong Meng, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Awis Qurni Sazili, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Thomas Schonwille
Associate Professor
Faculty of Veterinary Medicine
Utrecht University
The Netherlands
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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MAHDI EBRAHIMI

Date: 9 Novenber 2012

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